Measurement and Modulation of Cytokine Profiles Induced by Biomaterials

Washburn, NR; Weir, MD; Wang, FW; Bailey, LO
Polymers Division, National Institute of Standards and Technology, Gaithersburg, MD, 20899

Introduction: Tumor necrosis factor-α and interleukin-1β are cytokines of central importance in regulating inflammatory responses, and we have developed methods for quantifying inflammatory responses to materials in an *in vitro* model. Real time polymerase chain reaction (RT-PCR) was used to quantify changes in gene expression in Raw 264.7 macrophages and flow cytometry was used to measure apoptosis and necrosis. Together these techniques permit the investigation of mild and severe responses.

Cellular responses to a variety of materials were screened. These included poly(methyl methacrylate) (PMMA) and stainless steel particles and substrates, lipopolysaccharide, and photopolymerized dental resins.

Experimental: Upregulation of TNF-α and IL-1β expression was used as an early marker for inflammatory responses. RNA extraction for the RT-PCR experiments was carried out using an Rneasy Kit (Qiagen). The 18S gene was used as a housekeeping gene for gauging sample-to-sample variations in TNF-α and IL-1β expression. The following primers were used:

18S ribosomal subunit: 5' agcgaccaaaggaaccataa 3'
and 3' ctcctcctcctcctctctcg 5'
Tumor necrosis factor α: 5' tttcctcccaataccccttc 3'
and 3' agtgcaaaggctccaaagaa 5'
Interleukin-1β: 5' tgtgaaatgccacctttttga 3'
and 3' gtagctgccacagcttctcc 5'

Measurements were performed on a Bio-Rad iCycler using the QuantiTect SYBR Green RT-PCR Kit (Qiagen). Quantification of gene copy number was done using serial dilution standards of plasmid DNA containing the same sequences.

Cellular apoptosis and necrosis were markers for significant inflammatory responses. Flow cytometry was used to quantify the exposure of phosphatidyl serine in cell membranes to gauge early apoptosis and 7-AAD was used to measure membrane integrity as a test for late-stage apoptosis or necrosis.

Results: Representative data from the RT-PCR experiments are shown in Figure 1.

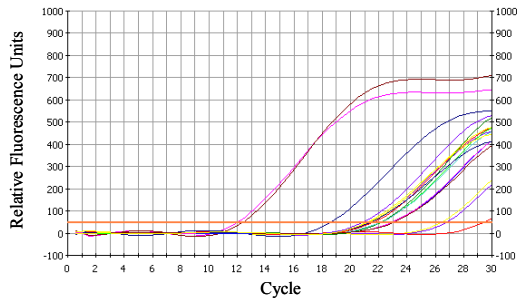

Figure 1: RT-PCR data from tests of PMMA particles.

Exposure of Raw 264.7 macrophages to PMMA particles for 8 h produced a 2.3-fold increase in TNF-α expression but an 87.9-fold increase in IL-1β expression. After 18 h, the TNF-a expression has returned to baseline levels while the IL-1b expression is still 45-fold larger than controls.[1] However, the macrophages exposed to PMMA particles showed very little evidence for apoptosis or necrosis, as measured by flow cytometry.

When exposed to stainless steel particles, macrophages displayed moderate upregulation of pro-inflammatory cytokines, but flow cytometry data reveal large numbers of apoptotic and necrotic cells. In Figure 2 are shown representative flow cytometry data from tests of stainless steel particles.

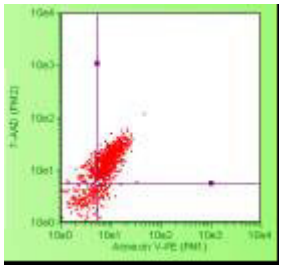

Figure 2: Flow cytometry data from macrophages exposed to stainless steel particles.

We hypothesize that cells in late apoptosis or necrosis were unable to efficiently participate in mounting inflammatory responses and were not able to produce significant cytokine levels.[2]

Similar results are seen for dental resins, where shorter photocuring times lead to large numbers of dying cells but lower levels of cytokine production.[3]

Conclusions: The expression profiles of TNF-α and IL-1β varied strongly depending on what materials were being exposed to the macrophages. Generally speaking, IL-1β upregulation lagged behind that of TNF-α, but then persisted for longer periods. Using RT-PCR we were able to quantify levels of cytokine production for macrophages exposed to a broad range of materials. Cytotoxic responses were measured using flow cytometry and found to provide complementary information to the data obtained from RT-PCR.

References:

[1] Bailey LO, Washburn NR, Simon CG, Chan ES, Wang FW. J Biomed Mater Res; *in press*.
[2] Bailey LO, Ridder S, Biancanello FS, Washburn NR. J Orthoped Res; submitted.
[3] Bailey LO, Weir MD, Washburn NR. J Dental Res; submitted.